CARNEGIE INSTITUTION OF WASHINGTON
DEPARTMENT OF GENETICS
COLD SPRING HARBOR, LONG ISLAND, N. Y.

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Dear Josh

Your letter came at a propitious time, as I have just come back from a visit to Yale, where the very problems you raise were fully aired, so that I have not only my own reactions, but that of the Tatum group. Lets take things one at a time: 1) with regard to the "Witkin" phenomenon, which is really the Ryan phenomenon -- I have recently talked with Ryan, and he has by now tested a large number of biochemical coli mutants, obtained from various sources, and has found that his dilution effect operates in a large number of same. I have examined about thirty strains which I isolated (but have not yet characterized) from B/r. Over half of these strains do the same thing, and <u>all</u> show evidence of considerable division on the minimal plate. Ryan has decided that the effect is due to division on the plate, with production of new reverse mutants (or supressors -- I do not have any way of distinguishing between them). With smaller inocula, individual cells go through a greater number of divisions than with larger inocula, so that the end number of bacteria on the plate tends to be similar regardless of the inoculum size, hence a fairly constant number of reverse mutations down the dilution series. I had independently arrived at the same type of explanation, based on microscopic observation of microcolony size, and some experiments involving washings from the plate which were then assayed. So, this does seem to be a rather general affair, though by no means necessarily universal. As to the implications for your recombination work, it seems ridiculous to me to consider this phenomenon, even if it applies to your mutants, as any kind of threat to your essential findings, and I

have made this clear to anyone who has raised the question. I don't want you to think that I have been party to any of the criticisms of your stuff on this score. It seems to me that the only possible effect that such a phenomenon might have on your results, if your mutants exhibit this behavior, is to distort the quantitative end of the linkage relations to some extent. For this reason only, I think it would be worth while, in view of Ryan's experiences and mine with regard to the generality of the phenomenon, for you to check your strains for this type of behavior. Otherwise, I simply don't see how your results can be affected. Finally, it is not clear whether the residual division of deficient strains on the minimal plates is due to presence of growth factor traces in the medium or in the cells, or to the rarity of true growth-factor dependence among these mutants. It wouldn't be easy to decide this. We have, however, (and so has Ryan presumably) used all the usual methods of washing the agar and the cells, and have found that intensifying our efforts to get rid of traces of growth factors does not reduce the residual growth.

All this makes our experiments with chemicals using reversions as an index of effects utterly worthless, since they were done with a strain exhibiting the dilution effect. The apparent effect was enormous, but similar increases could be obtained simply by diluting the control. While some strains may still prove workable, I 'm not hopeful, and have begun to play around with still other groups of mutations. No news yet on this score.

With regard to induced versus unmasked mutations, this is something I have given quite a lot of thought. First of all, we do not yet have any real evidence pro or con with respect to delayed effects produced by our chemicals. Full-scale work on this problem is just beginning, and we should

have an answer soon. There area number of reasons for believing that the unmasking notion is not a likely explanation, though it should be given some really thorough test. First of all, B and B/r differ very strikingly in the spontaneous incidence of "snakes" in fully grown cultures. B/r cells are largely binucleate, while cells having 4, 8, and many more nuclei are extremely frequent in B -- frequent enough so that one might expect a difference in the number of unmasked mutants between the two strains after a given treatment. Such difference is not observed. As a further check, I intend to induce really long, multinuclear snakes -- this can be done by such simple alterations of the medium as reducing the sugar concentration (Hinshelwood and Lodge), and does not require any drastic ultraviolation. Treatment of a population of uniformly long filamentous cells having twenty or so nuclei ought to help to give some evidence on this question. This will soon be done. The fact that the multinyclear condition is so common, and that there is so little evidence for segregation, makes one wonder if phage resistance may not be dominant. Another approach to the same problem would be to follow the change in number of mutants after break-up of filaments. U-v induced snakes divde up at a fairly sharp point, about three to four hours after irradiation. There ought to be a burst of mutants at this point, unless, of course, the binucleate condition is the ultimate unit. I don't really see how inhibition of nuclear division by the chemical can explain anything, as there is no appreciable cell division at all during treatment. It would have to be some differential destruction which would eliminate the covering action of one nucleus without actual separation into separate cells. This I find hard to visualize. It's certainly worth considering, though. I'm afraid I have a tendency to take criticisms so seriously that I spend most of my time building up my own confidence in the techniques, and very little time inducing mutations! Maybe it is better thus.

(over)

Thanks for writing – I always enjoy hearing from you. How goes your work?

My course in Genetics of Microorganisms has 40 students (I expected around 10) – it's lots of fun, but the large number makes it very hard to run as a seminar – as a result I have to do a lot of the talking myself.

How's Esther? Please give her my regards, and invite Cohen for set time.

Best regards,

Evelyn